Detection Of the Most Important Bacteria Accompanying Covid-19 Patients, with Measurement of Some Immube Indicators in Infected and Vaccinated Patients

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Abstract

Our study was conducted on (COVID19) patients lying in the isolation wards of Al-Sharqat-General-Hospital and Salah-Al-Din-Military-Hospital, for the period from mid-October 2021AD to March 2022AD, for ages (25-75years) and for both sexes. Collected 70-samples of throat swab, to detect secondary bacterial infection associated with COVID19, it was found that 66(94.28%) samples are growth-positive, and 4 (5.71%) samples are growth-negative. The isolates were distributed into 48 isolates (72.72%) belonging to Gram-positive bacteria, 18 isolates (27.27%) belonging to Gram-negative bacteria, and the number of pure single isolates reached 66 isolate. (24) isolates of Staphylococcus aureus with a percentage of 36.36% are the most prevalent bacteria among positive bacteria isolates. The rate of secondary bacterial infection among those infected with COVID-19 has been shown to be caused by Staph. aureus, which causes various respiratory infections, and (14) isolates of Streptococcus Pneumonia by 21.21% and it is among the bacterial causes associated with secondary infection, that these bacteria naturally inhabit the respiratory passages, but they can enter the lungs by inhalation, where it was found to be one of the most common causes of respiratory tract infection and (10) Streptococcus pyogens isolate in 15.15% is an associated bacterial cause with secondary infection. She indicated that the bacterium Strep. pyognes possess several virulence factors that help them spread by breaking down connective tissue. During the 1918 influenza outbreak, Pfeiffer discovered Strep. pyogenes as a prominent bacterial organism in influenza, as for the number of Gram-negative bacterial isolates , (14) isolates of Klebsiella pneumoniae amounted to 21.21% and it is the most Gram-negative bacterial species associated with secondary infection and antibiotic resistance with what It was reached (Ahmed et al, 2021) and in a percentage (40.5%), where these bacteria are transient natural flora present on the body, as it is considered an opportunistic infection in patients and hospitals. and (4) an isolate belonging to Moraxella catarrhalis with a percentage of 6.06%. The results showed the extent of resistance of some commonly used antibiotics against isolated bacteria, where Staphylococcus aureus was resistant to Azithromycin 100%, while Streptococcus pyogenes was more resistant to antibiotics with 100% to amoxicillin/clavulanic acid, levofloxacin, ceftriaxone, and amikacin. Pneumococci were 100% resistant to ceftriaxone, and as for Gram-negative bacteria, the results showed that they were resistant to most of the antibiotics used in the current study.

Keywords: COVID-19, secondary and co-infections with COVID-19, antibiotic resistance.

1. Introduction

COVID-19 posed a global health threat (Fisher et al, 2020). At the end of 2019, the new Corona virus was identified as the cause of a group of pneumonia cases in Wuhan, Hubei Province, China, and then spread rapidly, leading to the spread of the epidemic in All over China, an increasing number of cases followed in other countries around the world, (WHO, 2020a) as it spread rapidly to Thailand, Japan, South Korea, Singapore and Iran in the initial months (Sahu, 2020), where the World Health Organization declared a COVID-19 pandemic. The variation in clinical symptoms of COVID-19 is highly variable; Infection ranges from asymptomatic viral bacterial infection to acute, potentially fatal viral bacterial infection (Li et al. ، 2020). The main consequence of disease development in patients at later stages of infection is secondary bacterial infection. It was also found that at least one in seven patients with COVID-19 had a secondary bacterial infection with 50% of deaths during the SARS-CoV-2 epidemic caused by bacterial infection (Zhou et al. ، 2020). Untreated secondary, in most cases secondary bacterial infection develops in patients during or after the primary infection with an infectious disease, most often a virus (Morris et al, 2017; Wang et al, 2018). Almost all critically ill patients are treated with antibiotics to try to prevent secondary bacterial infection. (kash and Taubenberg-er, 2015; Macintyre et al, 2018) Co-infection is caused by multiple pathogens of viral, bacterial or fungal origin and occurs at the same time. (Jamison et al, 2010). Staphylococcus aureus and Streptococcus pneumoniae are the most common bacteria isolated during secondary infection (Handel et al, 2009).

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Many bacteria have acquired broad resistance to a range of antibiotics, and any increase in antibiotic use during the COVID-19 pandemic will have a detrimental effect on antibiotic resistance rates for bacterial pathogens (Reardon, 2020). Multidrug resistance (MDR) has become a global health challenge because in many cases there are no (chemical) antibiotics to treat such infections, including secondary infections. Several major human pathogens show antibiotic resistance including methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant streptococcus, Klebsiella, and carbapenem-resistant Pseudomonas aeruginosa (CRE), (Kumar and Chordia, 2017).

2. Material and Methods

70 swabs are collected from patients confirmed to be infected with the emerging coronavirus, COVID-19, for both sexes (males and females) and for ages 25-75 years from patients admitted to Al-Sharqat General Hospital and the Military Hospital in Tikrit by wearing a complete protective kit during the period from 1/12/2021 Until 1/3/2022, the samples are transferred directly to the laboratory.

Swabs are cultured on MacConkey agar medium and EMB medium for the growth of Gram-negative bacterial species, also grown on blood agar for the growth of bacterial species, grown on mannitol agar medium, salt and nutrient agar for growth of Gram-positive bacterial species, and incubated at 37 °C for 24-48 hours, to examine the secondary bacterial species associated with the emerging coronavirus COVID-19, the bacteria were diagnosed through a virtual examination and microscopy and through biochemical tests that included: the use of oxidase, catalase, Coagulase , In addition to the sensitivity test for Bacitracin and Optochin. A sensitivity test for all types of bacteria under study and to determine their resistance against 6 Antibiotics by tablet method of diffusion and based on (Kirby and Bauer 1966) which included (Amoxicillin + clavulanic acid) Gentamycin, Levofloxacin, Ceftriaxone, Azithromycin, Amikacin). The results were read by viewing the inhibitory areas around each disc and measuring the diameter in millimeters by a inserted ruler, and then the results were compared with the standard tables for the diameters of the antibiotics inhibition areas according to what was stated in (CLSI, 2022).

3. Resalts and Discusion

About 70 swabs were collected from the mouth and tonsils from patients who were confirmed to be infected with the new SARS-Cov-19 virus and who resided in isolation halls in hospitals (Al-Sharq Al-Alam Hospital, and the Military Hospital in Tikrit). It was found that 66 growth-positive samples of 94.28% and 4 growth-negative samples of 5.71% obtained 66 bacterial isolates. The isolates were distributed into 48 isolates (72.72%) belonging to Gram-positive bacteria, 18 isolates (27.27%) belonging to Gram-negative bacteria, and the number of pure single isolates reached 66 isolate. The results show isolates of positive bacteria shown in Figure (1), that (24) isolates of Staphylococcus aureus with a percentage of 36.36% are the most prevalent bacteria among positive bacteria isolates, where this result agrees with what was found (Elabadi et al, 2021). The rate of secondary bacterial infection among those infected with COVID-19 has been shown to be caused by Staph. aureus, which causes various respiratory infections, and (14) isolates of Streptococcus Pneumonia by 21.21% and it is among the bacterial causes associated with secondary infection, that these bacteria naturally inhabit the respiratory passages, but they can enter the lungs by inhalation, and these results are consistent with (Brueggemann et al, 2021), where it was found to be one of the most common causes of respiratory tract infection and (10) Streptococcus pyogens isolate in 15.15% is an associated bacterial cause with secondary infection, and these results are in agreement with what was found (Musher et al. , 2021). She indicated that the bacterium Strep. pyognes possess several virulence factors that help them spread by breaking down connective tissue. During the 1918 influenza outbreak, Pfeiffer discovered Strep. pyogenes as a prominent bacterial organism in influenza, as for the number of Gram-negative bacterial isolates , (14) isolates of Klebsiella pneumoniae amounted to 21.21% and it is the most Gram-negative bacterial species associated with secondary infection and antibiotic resistance with what It was reached (Ahmed et al, 2021) and in a percentage (40.5%), where these bacteria are transient natural flora present on the body, as it is considered an opportunistic infection in patients and hospitals. and (4) an isolate belonging to Moraxella catarrhalis with a percentage of 6.06% as shown in Figure (2).

The results showed that Staphylococcus aureus bacteria are resistant to several antibiotics including Levofloxacin, Ceftriaxone and Amikacin With a resistance ratio of 76.03%, 69.24% and 85.62% Respectively, the results also showed that the isolates of Staphylococcus aureus were multi-resistant to Azithromycin at a rate (100%), antibiotic resistance has become a serious threat to human health, and this may be due to the widespread and excessive use of antibiotics, as well as the lack of health awareness among members of the community (Urmi et al, 2021). Methylase 23s rRNA (23S) enzyme by genes (ermC, ermA, ermB) or by active efflux of antibiotics induced by ATP. The results of the current study showed that the bacteria Streptococcus pneumoniae are resistant to Azithromycin and Amoxillin\clavulanic antibiotics With a resistance ratio of 83.33% and 66.66%, respectively, and the results also showed that the isolates of Streptococcus pneumoniae were multi-resistant to ceftriaxone (100%). The development of antibiotic resistance is often associated with the misuse of prescribed antibiotics and their continued excessive use, with the results of the current study in agreement with those of (Dessie et al, 2021). The results showed that Streptococcus pyogenes resistance to Azithromycin was 66.66% In addition, the results showed that isolates of Streptococcus bacteria were resistant to other antibiotics 100% and included ceftriaxone, levofloxacin, amoxicillin/clavulanic acid, and Amikacin, where these results agree with what was found (Kebede et al, 2021), It's results showed that Streptococcus pyogenes is resistant to beta-lactam antibiotics due to the production of B-lactamase enzyme by Streptococcus pyogenes. The results of the study indicated that Klebsiella pneumoniae bacteria were resistant to the antibiotic Amoxicillin/Clavulanic acid by 100%, followed by Levofloxacin, Ceftriaxone and Azithromycin where the bacteria were resistant to 80%. The results of this study were consistent with a study by Chinese researchers (Xie et al, 2021). Gram-negative intestinal bacteria have also become resistant to beta-lactam antibiotics by producing beta-lactamases, especially AmpC, B-lactamase and ESBL. The study results also indicated that Moraxella bacteria were resistant to the antibiotics Ceftriaxone and Levofloxacin With a resistance ratio of 63.75, and 59.66 Respectively, while the antibiotics to which bacteria were resistant to Amoxic illin/Clavulanic acid was 100% and then Azithromycin where bacteria were resistant at 80%. Antibiotic resistance has become a serious threat to human health. This may be due to the widespread and excessive use of antibiotics As well as the lack of health awareness of community members as shown in shown in Table (1).

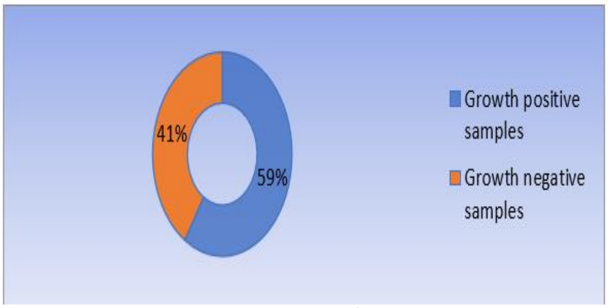

Figure (1): The percentage of positive & negative growth samples

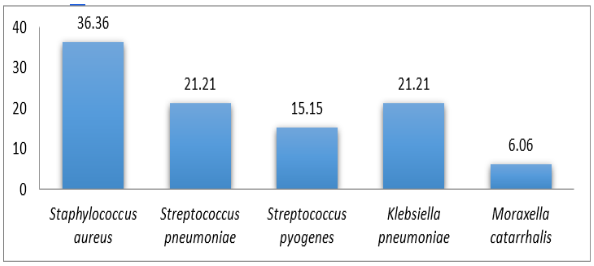

Figure (2) : Percentages of bacterial isolates isolated from COVID-19 patients

Table (1) : Resistance of bacterial species to antibiotics

AZM	AMC	CRO	LEV	CN	AK	Bacterial species
%100	%53.87	%30.86	%23.07	%30.77	84.62%	Staphylococcus aureus
%66.67	%100	%100	%100	--------	%100	Streptococcus pyogenes
%33.34	%83.34	%100	%50	--------	%66.67	Streptococcus pneumoniae
%80	%100	%20	%80	--------	%16.67	Klebsiella pneumoniae
%80	%100	%36.25	%59.67	--------	%36.34	Moraxella catarrhalis

4. Conclusions

The ultimate goal of research in the field of combined viral and bacterial infections of the upper respiratory tract is to translate our understanding of the molecular mechanisms that underlie this superinfection into the development of better diagnostics, treatment methods and preventive strategies. This is particularly important because we anticipate other epidemics and the emergence of new viruses in the future, and we anticipate the expansion of antibiotic resistance by bacterial pathogens, due to the misuse or overuse of antibiotics.

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